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# Interstitial Deletion of 2q Associated With Craniosynostosis, Ocular Coloboma, and Limb Abnormalities: Cytogenetic and Molecular Investigation

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**We report on the clinical and cytogenetic findings in a 9-year-old boy with a de novo deletion of 2q, shown by molecular analysis to have arisen from the paternal chromosome. Examination of microsatellite markers indicated deletion of bands 2q24.3 and 2q31. Clinical findings included craniosynostosis, bilateral ocular colobomata, and limb abnormalities, the latter being an emerging association with deletion of this region of 2q. Am. J. Med. Genet. 70:324–327, 1997.** © 1997 Wiley-Liss, Inc.

**KEY WORDS: craniosynostosis; ocular coloboma; syndactyly; deletion of chromosome 2q**

## INTRODUCTION

There have now been over 20 reported cases of deletions in 2q, the commonest single deletion being del(2)(q31q33), with 14 cases in the literature. The clinical characteristics of individuals with del(2)(q31q33) have been reviewed by Ramer et al. [1989, 1990]. A smaller number of patients have been reported with del(2)(q24q31) [Wamsler et al., 1991]; recently Boles et al. [1995] have drawn attention to the association of this deletion with digital anomalies. We report the clinical and molecular findings in a new patient now aged 9 years, with a deletion of chromosome 2 apparently involving bands q24 and q31, which confirms and extends this association.

## CLINICAL REPORT

The affected boy (Fig. 1a,b) was born after 37 weeks of gestation to a 34-year-old woman previously gravida 2 para 2, with no significant personal medical history. The father of all three children was 36 years old at the time of delivery of the patient reported here, and has type I diabetes. Their first son is mildly mentally retarded but has no anomalies, there was a significant history of birth asphyxia and his chromosomes are normal. Their second son is normal. The third pregnancy was uncomplicated, but the mother had an amniocentesis at 17 weeks to screen for the inheritance of a paternal marker chromosome. The amniotic fluid sample was reported as normal, excluding both trisomy 21 and inheritance of the paternal marker chromosome. The delivery was normal, Apgar scores were 3 at 1 minute and 8 at 5 minutes, the birth weight was 2,060 g and head circumference was 30.5 cm (both just beneath the third centile). When examined at age 4 days, the following findings were noted: a high prominent forehead, flat occiput, flat nasal bridge, low set ears, small palpebral fissures, small mandible, high palate, and hypertelorism with an antemongoloid slant and proptosis (Fig. 1a). Examination of his eyes showed bilateral colobomata of the iris, retina, and optic nerve. He had bilateral single palmar creases, camptodactyly of the thumbs, and clinodactyly of the 5th fingers. There was syndactyly of toes 3 to 5 on the right and 2 to 5 on the left, with a wide cleft between the 1st and 2nd toes (Fig. 1b). Abdominal organs were normal apart from cryptorchidism.

Initially he was well but at 14 days he developed a heart murmur and heart failure, which was managed with digoxin and frusemide; the heart failure resolved over a few months. An echocardiogram demonstrated a small atrial septal defect, small ventricular septal defect, small patent ductus arteriosus, and possible bicuspid pulmonary valve. Up to 11 months his head circumference continued to grow just below the third centile, but at 13 months it was noted that his head had stopped growing. A CT head scan showed no abnormality of the brain, but skull radiographs demonstrated that the upper part of the right coronal suture was present but "petered out" inferiorly, the left coronal suture and the sagittal suture were absent, and the metopic suture was present but deviated to the left. Progressive proptosis secondary to his craniosynostosis

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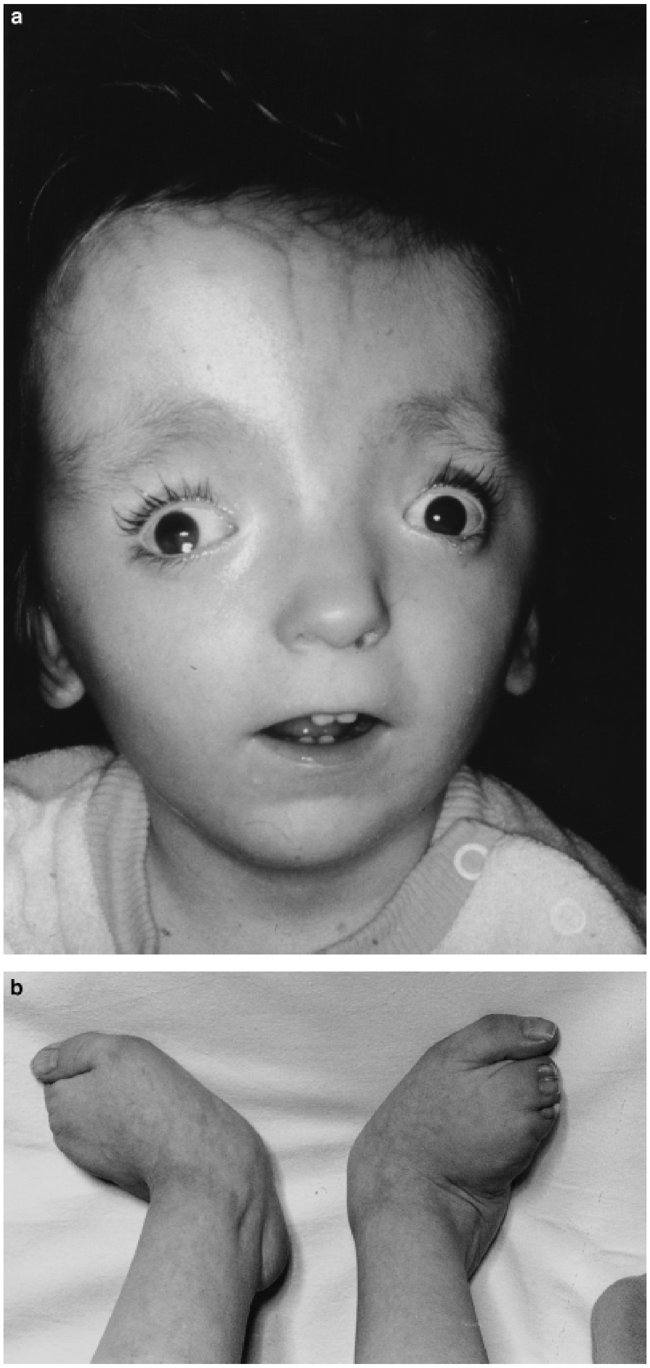


Fig. 1. **a:** The patient at age 2.5 years (before cranial surgery). **b:** The patient's feet at age 4 years, demonstrating syndactyly of toes 3–5 on the right and 2–5 on the left.

led to the development of corneal ulcers; a frontal advancement was performed at the age of two and a half years to improve eye closure. He developed joint contractures during the first two years of life, thought to be secondary to disuse. At three years he underwent herniotomy and orchidopexy for bilateral inguinal herniae. He is severely mentally retarded: he started walking at 5 years, he says "mum" but has no two syllable babble, and remains doubly incontinent. His height has remained persistently just below the 3rd centile.

## CYTOGENETIC AND MOLECULAR ANALYSIS

Lymphocytes were cultured with thymidine synchronization and G banded by trypsin, according to standard protocols. Fluorescent in situ hybridization (FISH) studies were undertaken using digoxigenin labelled acrocentric beta satellite probe (Oncor, Gaithersburg, MD) and chromosome 2 alpha satellite probe (Oncor) with fluorescein isothiocyanate (FITC) visualization according to standard procedures.

DNA was extracted by standard methods. Primers for all of the microsatellite markers are described by Gyapay et al. [1994]. PCR was carried out on a Hybaid Omnigene thermal cycler using microtitre plates. The reaction volume was 15 μl with 80 ng DNA, 0.1 mM dNTPs, 200 nM forward and reverse primers, and 0.4 units Amplitaq (Perkin Elmer). Buffer conditions were 10 mM Tris HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and PCR conditions were (94°C 4 minutes, 55°C 1 minute) × 1, (94°C 1 minute, 55°C 1 minute) × 35, (94°C 1 minute, 55°C 1 minute, 72°C 10 minutes) × 1 with the steps from 55°C to 94°C ramped at 1°C/second. The PCR product was heated to 96°C and electrophoresed on a 6% denaturing polyacrylamide gel, blotted onto Hybond N+ nylon membrane, probed with a $(CA)_{10}$ oligonucleotide labelled with $^{32}$P-dCTP using terminal transferase, and visualised by autoradiography. The physical location of the microsatellite markers was deduced by integrating the genetic maps of these markers [Gyapay et al., 1994] with markers whose physical location is given in the Genome Database.

The patient was referred at the age of 8 years for detailed cytogenetic analysis which showed an interstitial deletion of the long arm of chromosome 2. The parents and brothers had normal chromosomes, apart from an additional marker chromosome present only in the father and paternal grandfather, which was absent in the proband. Cytogenetic and FISH studies identified the familial marker as a bisatellited dicentric chromosome of acrocentric origin and, therefore, unlikely to be associated with the origin of the chromosome 2 deletion. Cytogenetic analysis showed loss of band q31 but the exact breakpoints could not be defined as the banding pattern could be the product of several possible combinations of breakpoints occurring in bands q24–q32 (Fig. 2).

Further delineation of the breakpoints was therefore undertaken by microsatellite analysis. DNA samples from the propositus and both parents were analysed for the segregation of polymorphic microsatellite markers mapping to 2q (Table I). Three markers demonstrated hemizygosity in the proband and indicated that the deletion was of paternal origin and extended over a genetic distance of at least 13 and at most 25 cM (Table I and Fig. 3). Correlation of the genetic location of physically mapped DNA markers indicated that the deletion included at least part of bands 2q24.3 and 2q31, and possibly part of 2q32 (Fig. 3). The patient's karyotype is, therefore, most likely 46,XY,del(2)(q24.3q32.1).

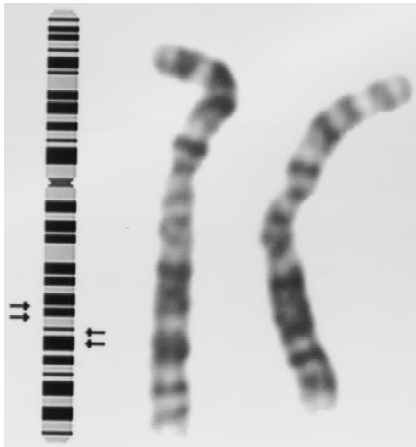

Fig. 2. Chromosome 2 pair from patient (deleted chromosome on right). Ideogram with paired arrows illustrate regions of possible breakpoints to give similar G banding pattern. The proximal breakpoint (shown to the left of the ideogram) lies between 2q24.2 and 2q31.1; the distal breakpoint (shown to the right) lies between 2q31.3 and 2q32.3.

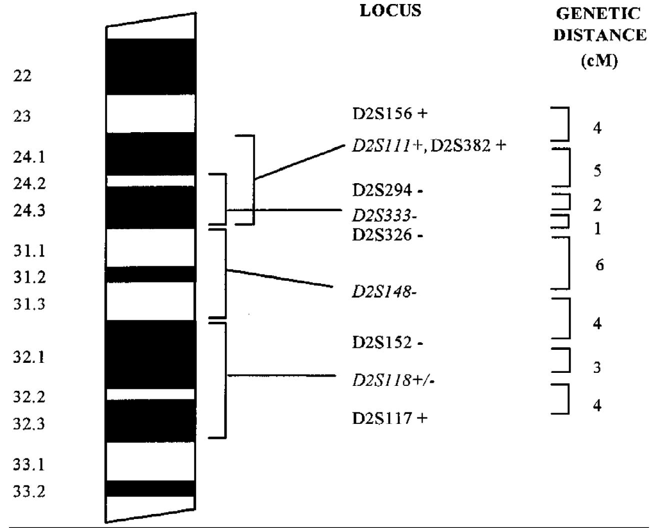


Fig. 3. Correlation of genetic and physical maps for chromosome bands 2q22–q33.2. Markers used in the analysis of the family are shown in upright text; markers whose physical locations are known are shown in italic text. The order and genetic distance between these markers is shown in centiMorgans [Gyapay et al., 1994]. Deletion status of physically mapped markers in the proband was deduced from adjacent genotyped markers (+, not deleted; –, deleted; +/–, status ambiguous).

## DISCUSSION

We have reported a case of de novo deletion of chromosome 2q, including bands 2q24.3 and 2q31. The phenotypic findings in our case match closely those with del(2)(q31q33) reviewed by Ramer et al. [1989,1990], with growth failure, minor facial anomalies (but no cleft palate, which has been reported in 6/14 cases), coloboma of iris, retina, and optic nerve (coloboma of iris has been reported in one case by Young et al. [1983]), heart defects, and limb abnormalities. The main finding that distinguishes our case from those previously reported is the presence of craniosynostosis, which has not been previously reported in association with deletion of this region of chromosome 2.

The limb abnormalities in our case were camptodactyly and fifth finger clinodactyly in the hands and syndactyly of the toes (3 to 5 on the right and 2 to 5 on the left) with a wide cleft between the first and second toes. This pattern of abnormalities, with the feet more severely affected than the hands, is typical of those seen in the reported cases of del(2)(q31q33), and has been reported in cases of del(2)(q24q31) [Wamsler et al., 1991; Boles et al., 1995]. Boles et al. [1995] have suggested that the 2q31.1 segment is critical in the development of the specific limb defects seen in these patients, and our case adds further weight to this.

None of the previously reported cases of de novo deletion of this region of chromosome 2 was analysed for the parental origin, although Ramer et al. [1990] reported 5 cases arising in the offspring of a male carrier of a dir ins (6;2)(q16;q31q33). Molecular analysis demonstrated that in our case the deletion arose de novo on the paternal chromosome.

Cytogenetic analysis alone could not determine the exact breakpoints and required supplementary molecular investigations to define the chromosome 2 deletion. In view of several different combinations of breakpoints producing a visually similar banding pattern, caution should be taken when comparing phenotypes of other previously reported cases with apparently identical deletions. Careful molecular study of more cases with chromosomal rearrangements in this region is required [Sanchez and Goldschmidt, 1994].

Among genes which may map to the deleted region, those most likely to contribute to the development of the malformations described here include the HOXD/EVX2 cluster (2q31), the integrins ITGA4 and ITGA5 (2q31–q32), and the distal-less homeobox genes DLX1 and DLX2 (2q32). Boles et al. [1995] excluded deletion of the HOXD13 or EVX2 in their patient with del(2)(q24q31), suggesting that haploinsufficiency of these genes does not contribute to the limb abnormalities, although a gain of function mutation of the HOXD13 gene was recently reported in type II synpolydactyly [Muragaki et al., 1996]. The most closely homologous regions in the mouse genome are 1A5–B, 2C–D, and 2E4–F. A mouse (del(2)59H) has recently been described with an induced deletion (2E2–5) which spans part of this region [Burtenshaw et al., 1995]. The phenotype of this mouse includes small size, a slightly domed short head, and hyperpigmentation of the genitalia; its viability is about 70%. No other fertile mice with definite deletions in the regions of conserved synteny have been described (E.P. Evans, personal communication).

TABLE I. Inheritance of Microsatellite Markers From Chromosome 2q*

| | Genotype | | | |
|---|---|---|---|---|
| Locus | Mother | Father | Propositus | Interpretation |
| D2S156 | 1,2 | 1,3 | 1,3 | Not deleted |
| D2S382 | 1,2 | 1,3 | 1,2 | Not deleted |
| D2S294 | 1,2 | 3,4 | 1,– | Paternal deletion |
| D2S326 | 1,2 | 2,3 | 1,– | Paternal deletion |
| D2S152 | 1,2 | 2,3 | 1,– | Paternal deletion |
| D2S117 | 1,2 | 1,3 | 1,3 | Not deleted |

*The markers are ordered from centromere (top) to telomere (bottom).

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